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Mesophilic and Psychrotrophic Bacteria from Meat and Their Spoilage Potential In Vitro and in Beef[▽]

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Mesophilic and psychrotrophic populations from refrigerated meat were identified in this study, and the spoilage potential of microbial isolates in packaged beef was evaluated by analyzing the release of volatile organic compounds (VOC) by gas chromatography-mass spectrometry (GC/MS). Fifty mesophilic and twenty-nine psychrotrophic isolates were analyzed by random amplified polymorphic DNA-PCR, and representative strains were identified by 16S rRNA gene sequencing. *Carnobacterium maltaromaticum* and *C. divergens* were the species most frequently found in both mesophilic and psychrotrophic populations. *Acinetobacter baumannii*, *Buttiauxella* spp. and *Serratia* spp. were identified among the mesophilic isolates, while *Pseudomonas* spp. were commonly identified among the psychrotrophs. The isolates were further characterized for their growth at different temperatures and their proteolytic activity in vitro on meat proteins extracts at 7°C. Selected proteolytic strains of *Serratia proteamaculans*, *Pseudomonas fragi*, and *C. maltaromaticum* were used to examine their spoilage potential in situ. Single strains of these species and mixtures of these strains were used to contaminate beef chops that were packed and stored at 7°C. At time intervals up to 1 month, viable counts were determined, and VOC were identified by GC/MS. Generally, the VOC concentrations went to increase during the storage of the contaminated meats, and the profiles of the analyzed meat changed dramatically depending on the contaminating microbial species. About 100 volatiles were identified in the different contaminated samples. Among the detected volatiles, some specific molecules were identified only when the meat was contaminated by a specific microbial species. Compounds such as 2-ethyl-1-hexanol, 2-buten-1-ol, 2-hexyl-1-octanol, 2-nonanone, and 2-ethylhexanal were detectable only for *C. maltaromaticum*, which also produced the highest number of aldehydes, lactones, and sulfur compounds. The highest number of alcohols and ketons were detected in the headspace of meat samples contaminated by *P. fragi*, whereas the highest concentrations of some alcohols, such as 1-octen-3-ol, and some esters, such as isoamyl acetate, were produced by *S. proteamaculans*. In conclusion, different microbial species can contribute to meat spoilage with release of different volatile compounds that concur to the overall quality decrease of spoiling meat.

The storage of food using low temperatures is by far the most commonly used measure of preservation in fresh foods production and distribution. Meat is recognized as one of the most perishable foods, and refrigeration temperatures are always used to delay the spoilage of fresh meat. The low temperatures are used for the storage of carcasses after slaughter, subprimal cuts and during transport of meat to the distributors, and finally for the storage of meat at the various retail sites. During all of these phases the chill temperature is used to delay meat alteration through the development of spoilage bacteria.

The development of organoleptic spoilage is related to microbial consumption of meat nutrients, such as sugars and free amino acids and the release of undesired volatile metabolites. In chill-stored meat these activities may be performed at low temperatures by psychrotrophic bacteria, compromising the sole effect of temperature as affecting preservation.

Different spoilage species and strains can colonize the meat surface through different stages involving adsorption to the meat surface (7, 18) and attachment by glycocalix formation (9). The development of these phases depends on the intrinsic and extrinsic ecological factors of a particular meat ecosystem such as pH, meat surface morphology, O_2 availability, the presence and development of other bacteria and, most of all, temperature (10, 11, 19, 26, 28, 32, 33, 47, 49).

Many groups of organisms contain members potentially contributing to meat spoilage under appropriate conditions. This makes the microbial ecology of spoiling raw meat very complex, and thus the spoilage is very difficult to prevent.

Bacteria developing on meat at chill temperatures are regarded as psychrotrophic. They belong to microbial genera of both gram positive, such as lactic acid bacteria, and gram-negative bacteria, such as *Pseudomonas* spp. and *Enterobacteriaceae* (21, 24). Species of *Pseudomonas* are particularly involved in the spoilage of meat stored at chill temperatures (15, 30, 33). In particular, the microflora of vacuum packaged chill-stored meat is characterized in most cases by psychrotrophic lactic acid bacteria (4, 10, 23, 48, 54, 55).

Spoilage development is a complicated biological event, which needs to be studied at the species and biotype level. Certain microbial taxa may be differently influenced by the specific storage conditions, and different microbial species may unpredictably develop during meat storage, thus influencing the time and type of spoilage development (14). There is still

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a need to assess, within each spoilage group, which species are actually involved in the spoilage of meat. Such a role in chilled-meat spoilage strongly depends on the spoilage activities of psychrotrophic bacteria capable of growing in meat releasing undesired metabolic compounds mainly arising from nutrient degradation at low temperatures. In addition, studies on the correlation between spoilage-related molecules release and development of specific microbial species during meat storage are still not available.

The aim of the present study was to describe the microbial diversity of mesophilic and psychrotrophic bacteria isolated from meat and to investigate the spoilage potential of the identified microbial taxa both in vitro and in packaged beef chops.

MATERIALS AND METHODS

Meat samples and isolation of bacteria. In order to assess the diversity of mesophilic and psychrotrophic bacteria from meat and to have a source of isolation of strains to be evaluated for their spoilage potential, four portions (500 g each) of vacuum-packed (polyamide [20 μm]-polyethylene [70 μm]; oxygen permeability, 2 $cm^3\ m^{-2}\ h^{-1}$ at 23°C [provided by Alpak S.r.l., Milan, Italy]) beef muscles (*Longissiumus dorsi*) from a slaughterhouse were used for the viable counts and isolation after storage for 10 days at 7°C. Surface samples (~35 cm^2) from each muscle were aseptically weighed and homogenized in 225 ml of one-quarter-strength Ringer's solution (Oxoid) for 2 min in a stomacher (LAB blender 400; PBI, Italy) at room temperature. Decimal dilutions were prepared in the same solution, and aliquots of 0.1 ml of the appropriate dilutions were plated in triplicate on plate count agar (PCA; Oxoid) and incubated aerobically at 30°C for 72 h and 7°C for 10 days for the enumeration of mesophilic and psychrotrophic bacteria, respectively. After viable counts, colonies present on the countable plates were randomly isolated and purified on PCA plates. The purified isolates were preliminary characterized by microscopic observations and Gram, catalase, and oxidase reactions. Working cultures were maintained in tryptic soy broth with 25% glycerol at −20°C.

PCR amplifications. DNA extraction from 79 isolates was carried out with a loopful of grown culture on PCA according to the method of Marmur (40). After the extraction, the DNA was quantified by measuring the absorbance (260 nm) by a spectrophotometer (DU640; Beckman Instruments, Inc., Fullerton, CA). In order to reduce the number of isolates to be identified by 16S rRNA gene sequencing, the DNA extracted was subjected to random amplified polymorphic DNA (RAPD)-PCR for biotype screening among the isolates, and then the 16S rRNA gene of different biotypes was sequenced for taxonomic identification.

The primer XD5 (5′-CTGGCGGCTG-3′) was used for RAPD-PCR analysis; previous experiments had shown good discrimination power of this primer for strains of the same species of both yeast and bacteria (12, 16, 17). PCRs were carried out in 25 μl of reaction mix containing 1× *Taq* polymerase buffer (Invitrogen, Milan, Italy), 2.5 mM $MgCl_2$, each deoxynucleoside triphosphate at a concentration of 0.4 mM, a 1.08 μM concentration of the primer, 2.5 U of *Taq* polymerase, and 20 ng of the extracted DNA. PCR was carried out in a programmable heating incubator using an initial denaturation step at 94°C for 1 min, followed by 40 cycles of 1 min at 94°C, 1 min at 31°C, and 2 min at 72°C per cycle. Finally, a 7-min extension period at 72°C was performed. The amplified products (25 μl) were resolved by electrophoresis on 1.5% (wt/vol) agarose-Tris-borate-EDTA at 7 V cm^{-1} for 2.5 h. A 1-kb DNA ladder (Invitrogen) was used as a molecular weight marker.

In order to amplify the 16S rRNA gene, the oligonucleotide primers described by Weisburg et al. (63) fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′-AAGGAGGTGATCCAGCC-3′) (*Escherichia coli* positions 8 to 17 and 1540 to 1524, respectively) were used. The PCR mixture (final volume, 50 μl) contained 1 μl (20 ng) of template DNA, each primer at a concentration of 0.2 μM, each deoxynucleoside triphosphate at a concentration of 0.25 mM, 2.5 mM $MgCl_2$, 5 μl of 10× PCR buffer, and 2.5 U of *Taq* polymerase (Invitrogen). PCR conditions consisted of 30 cycles (1 min at 94°C, 45 s at 54°C, and 2 min at 72°C) plus one additional cycle at 72°C for 7 min as a final chain elongation.

16S rRNA gene sequencing. PCR products from the 16S rRNA gene (1.6 kb), obtained from isolates, were purified by using a QIAquick PCR purification kit (Qiagen) according to the supplier's instructions. The DNA sequence was determined by the dideoxy chain termination method by using the primer couple above described (63). Research for DNA similarity was performed with the National Centre of Biotechnology Information GenBank (1).

Growth at different temperatures. Representative strains of the different RAPD-PCR biotypes were characterized by their ability to grow at different temperatures. The test was performed by streaking a microbial suspension (with an optical density at 600 nm of 0.3) on PCA plates. Three plates were inoculated with each culture, followed by incubation at 7, 20, or 30°C. Growth was evaluated by observing a full biomass development along the streaking point.

Proteolytic activity in vitro at 7°C. The proteolytic activity of the strains was determined after growth on agar media containing meat myofibrillar protein extracts. Lean minced beef (20 g) was diluted 1:10 (wt/vol) with 20 mmol of phosphate buffer (pH 6.5) $liter^{-1}$, homogenized in a Stomacher 400 blender for 3 min, and centrifuged at 16,000 × *g* for 20 min at 4°C. The resulting pellet was used for myofibrillar preparation as described by Sanz et al. (53). It was resuspended in 200 ml of 0.03 mol of phosphate buffer (pH 7.4) $liter^{-1}$ containing 0.1% (vol/vol) Triton X-100 and homogenized for 2 min in a Stomacher 400 blender. After centrifugation at 16,000 × *g* for 20 min at 4°C, the pellet was washed three times by resuspension in the same buffer to remove muscle proteases. The resulting pellet was weighed and resuspended in 9 volumes of 0.1 mol of phosphate buffer (pH 6.5) $liter^{-1}$ containing 0.7 mol of KI $liter^{-1}$. After Stomacher homogenization for 8 min, the suspension was centrifuged at 10,000 rpm for 20 min at 4°C, and supernatant was diluted with water 10 times to avoid possible inhibition of bacterial proteases by the presence of KI. The extract was standardized at 5 mg of protein ml^{-1} by using a Bradford protein assay (Bio-Rad, Milan, Italy), filter sterilized (0.22-μm pore size), and added at concentration of 1 mg ml^{-1} to an agar medium consisting of 0.5% tryptone, 0.25% yeast extract, 0.1% glucose, and 1.5% agar (pH 6.9) and then poured into petri dishes. After solidification of the medium, 50 μl of a 24 h culture broth of each strain were spotted in wells bored in the agar plates that were incubated at 7°C for 1 week. After incubation, the agar plates with proteins were removed from petri dishes and stained for 5 min in 0.05% (wt/vol) Brilliant Blue R (Sigma) in methanol-acetic acid-distilled water (50:10:40). After destaining in methanol-acetic acid-water (25:5:70), the presence of a clear zone surrounding the inoculated wells indicated proteolytic activity (42).

Meat contamination and storage under vacuum at 7°C. The strains *Serratia proteamaculans* 42M, *Pseudomonas fragi* 25P, and *Carnobacterium maltaromaticum* 9P were selected for their proteolytic activity at 7°C as described above described and used for the spoilage assays in vacuum packaged meat as follows. An entire muscle (*Longissimus dorsi*) was surface sterilized by flaming, and the external part was removed by a sterile bistoury in order to achieve a superficial decontamination. Cubical meat chops (~40 g) were cut from the internal part of the muscle and were spiked with a strain suspension in 200 ml of quarter-strength Ringer's solution (Oxoid) of the above strains at a concentration of 10^7 CFU ml^{-1}. The contamination was performed by using the above strain singly or a combination of the three strains at the same concentration; uninoculated meat chops were included as a control. The chops were dried on a sterile cloth for 1 min, vacuum packed in a plastic film bag (coextruded, low-density polyethylene [12 μm] and ethyl vinyl alcohol [95 μm]; oxygen transmission rate, 0.30 $cm^3\ m^{-2}\ h^{-1}$ at 23°C) and stored at 7°C for 30 days. The meat chops were packed singly; three packages were opened for microbiological and three for chemical analyses, respectively, at each time of sampling.

After 0, 5, 15, and 30 days of storage, samples from each package were aseptically weighed and homogenized in quarter-strength Ringer's solution (Oxoid) for 2 min in a stomacher (LAB blender 400; PBI, Italy) at room temperature. Decimal dilutions were prepared in Ringer's solution, and aliquots of 0.1 ml of the appropriate dilutions were spread in duplicate on the following media: PCA (Oxoid) incubated at 20°C for 48 h for the enumeration of total aerobic bacteria; violet red bile glucose agar (Oxoid) for the *Enterobacteriaceae*, incubated at 30°C for 24 to 48 h; CTSI agar (62) incubated at 20°C for 1 week; and *Pseudomonas* agar with cetrimide-fucidin-cephaloridine selective supplement (Oxoid) for *Pseudomonas*, incubated at 25°C for 48 h. The results were calculated as the mean log CFU/g for three determinations. The data of the three repeated trials were analyzed by analysis of variance (Systat Software, v. 5.2.1 for Mac) in order to verify the differences between the samples during the storage.

VOC determination by GC/MS. Chemical analyses were performed after 0, 15, and 30 days of storage. A headspace (HS) solid-phase microextraction (SPME) analysis was carried out on 5 g of meat from the chopped samples; the meat was homogenized in 100 ml of water, and 30 g of NaCl and placed in a hermetically closed vial (150 ml). The fiber (CAR/DVB/PDMS; Supelco Sigma-Aldrich, Bornem, Belgium) was immersed in the HS for 1 h at room temperature. Thermal desorption of the analytes from the fiber inside the GC injection port was carried out in the split mode (1/10) at a desorption temperature of 250°C for 1 min. All samples were analyzed with an HP6890 coupled to a 5973N quadru-

TABLE 1. Mesophilic bacteria isolated in this study[a]

Strain	RAPD profile	No. of isolates	Closest relative	% Similarity	Closest relative accession no.	Growth (no. of days) at[b]:			Proteolytic activity at 7°C
						7°C	20°C	30°C	
30M	M	1	*Acinetobacter baumannii*	99	X81667	5	1	1	–
46M	U	1	*Acinetobacter baumannii*	99	X81667	4	1	1	–
47M	V	1	*Acinetobacter baumannii*	100	X81667	4	1	1	–
41M	R	1	*Buttiauxella agrestis*	98	AJ233400	2	1	1	–
43M	T	3	*Buttiauxella agrestis*	100	AJ233400	5	1	1	–
52M	a	1	*Buttiauxella gaviniae*	98	AJ233403	4	1	4	–
51M	Y	1	*Buttiauxella noackiae*	98	AJ293687	4	1	4	–
1M	B	3	*Carnobacterium divergens*	99	AM179875	5	1	1	–
5M	C	1	*Carnobacterium divergens*	99	AM179875	5	2	2	–
17M	F	4	*Carnobacterium divergens*	98	AY543036	5	2	1	+
13M	I	3	*Carnobacterium divergens*	98	AM179875	5	2	2	–
14M	G	11	*Carnobacterium divergens*	99	AM179875	5	2	1	–
29M	L	1	*Carnobacterium divergens*	99	AY543036	5	1	1	–
2M	A	3	*Carnobacterium maltaromaticum*	98	AY543035	5	1	1	–
7M	E	1	*Carnobacterium maltaromaticum*	98	AY543030	5	1	1	–
6M	D	2	*Carnobacterium* spp.	98	DQ343755	5	2	2	–
53M	b	1	*Hafnia alvei*	99	AY253922	4	1	1	–
18M	H	1	*Lactobacillus sakei*	98	AY204898	5	1	1	–
39M	Q	2	*Pseudomonas putida*	99	AB294558	1	1	1	–
37M	P	1	*Serratia proteamaculans*	98	AJ233435	1	1	1	–
42M	S	1	*Serratia proteamaculans*	100	AJ233434	2	1	1	+
34M	O	3	*Serratia* spp.	99	DQ103511	5	1	1	–
48M	Z	1	*Serratia* spp.	99	DQ103511	4	1	1	–
49M	W	1	*Stenotrophomonas maltophilia*	99	AY486381	4	1	1	+
32M	J	1	*Streptococcus parauberis*	99	AY942570	4	4	4	–

[a] RAPD-PCR profile, species identification by sequencing of the 16S rRNA gene, growth at different temperatures, and proteolytic activity at 7°C are indicated.
[b] Number of days to obtain growth on PCA at a defined temperature.

pole HP mass spectrometer (Agilent Technologies, Santa Clara, CA). The gas chromatograph was equipped with an HP-5ms capillary column (30 m by 0.25 mm [inner diameter]), and the carrier gas used was helium (1 ml min^{-1}). For the analysis of volatile compounds the GC oven temperature was programmed from 40°C (held for 7 min) to 180°C at 5°C/min. The masses were scanned on *m/z* range of 45 to 350 amu. For the identification of volatile components, the NIST library and/or comparison with spectra and retention times of standards were used.

The quantitative determinations were carried out through calibration in the matrix in the range of verified linearity; multiple replicates (n = 3 to 6) of the samples were analyzed.

RESULTS

Isolation and identification of mesophilic and psychrotrophic bacteria from meat. The eight meat samples stored for 10 days under vacuum showed viable counts determined on PCA ranging from 7.0×10^5 to 5.8×10^6 CFU g^{-1} for mesophilic bacteria and from 1.1×10^6 to 2.5×10^7 CFU g^{-1} for psychrotrophic bacteria. The number of psychrotrophic bacteria was, on average, 1 log cycle higher than mesophilic bacteria in each sample. After purification and preliminary characterization, 79 isolates were subjected to DNA extraction and RAPD-PCR typing in order to identify copies of the same strains and reducing the number of isolates to identify by 16S rRNA gene sequencing. A total of 50 mesophilic and 29 psychrotrophic isolates were analyzed by RAPD-PCR, obtaining 25 different profiles for mesophilic (Table 1) and 19 for psychrotrophic bacteria (Table 2). One representative strain for each RAPD profile was identified by 16S rRNA gene sequencing, and the results are shown in Tables 1 and 2. The mesophilic bacteria belonged to 15 species of nine different genera (Table 1), while 10 species of eight genera were identified among the psychrotrophic bacteria (Table 2). *Carnobacterium maltaromaticum* and *C. divergens* were the species most frequently found in both mesophilic and psychrotrophic populations. *Acinetobacter baumannii*, *Buttiauxella* spp. and *Serratia* spp. were also identified among the mesophilic isolates (Table 1), while *Pseudomonas* spp. were commonly identified among the psychrotrophs (Table 2). Remarkably, more RAPD biotypes of the same species were found among the isolates of the same group. In addition, strains of the same species found both in mesophilic and psychrotrophic bacteria displayed completely different RAPD-PCR profiles (Tables 1 and 2).

Growth trials and proteolytic activity. The results of growth at different temperatures of mesophilic and psychrotrophic bacteria are reported in Tables 1 and 2, respectively. Most of carnobacteria grew at 7°C in 5 days—both the strains isolated as mesophilic bacteria and those isolated as psychrotrophic bacteria. However, the strains isolated as psychrotrophic grew very slowly at 30°C compared to the mesophiles that could grow in 24 to 48 h at the same temperature (Tables 1 and 2). *Serratia proteamaculans* strains 37M and 42M grew at 7°C in 1 and 2 days, respectively. Almost all of the mesophilic bacteria grew in 4 to 5 days at 7°C but took only 24 h to grow at 20 and 30°C (Table 1). In contrast, most of the psychrotrophic *Pseudomonas* strains could grow in 3 days at 7°C but showed no growth at 30°C in 10 days (Table 2).

The results of the proteolytic activity at 7°C tested on agar plates containing a microfibrillar beef extract are also reported in Tables 1 and 2. Only *C. divergens* 17M, *S. proteamaculans* 42M, and *Stenotrophomonas maltophilia* 49M among the meso-

TABLE 2. Psychrotrophic bacteria isolated in this study[a]

Strain	RAPD profile	No. of isolates	Closest relative	% Similarity	Closest relative accession no.	Growth (no. of days) at[b]:			Proteolytic activity at 7°C
						7°C	20°C	30°C	
14P	t	1	*Brochothrix thermosphacta*	98	AY543029	3	1	4	–
21P	e	1	*Buttiauxella agrestis*	98	AJ293686	3	1	1	–
3P	p	4	*Carnobacterium divergens*	100	AM179875	5	3	4	–
5P	q	4	*Carnobacterium divergens*	99	AM179875	5	2	4	–
16P	u	4	*Carnobacterium divergens*	99	AM179875	5	3	5	–
2P	o	1	*Carnobacterium maltaromaticum*	100	AY543034	4	2	4	–
9P	s	2	*Carnobacterium maltaromaticum*	100	AY543034	5	3	–	+
19P	z	1	*Halomonas* spp.	98	DQ129699	3	2	1	–
17P	v	1	*Leuconostoc gelidum*	98	AB022921	5	8	3	–
25P	i	1	*Pseudomonas fragi*	98	AF094733	3	1	–	+
23P	g	1	*Pseudomonas* spp.	99	AY569287	3	3	–	–
24P	h	1	*Pseudomonas* spp.	99	AY569287	3	1	5	–
26P	j	1	*Pseudomonas* spp.	98	DQ084461	3	1	–	–
27P	k	1	*Pseudomonas* spp.	98	DQ084461	3	2	–	–
28P	l	1	*Pseudomonas* spp.	99	DQ084461	3	1	–	+
29P	m	1	*Pseudomonas* spp.	99	AY303304	3	2	–	–
30P	n	1	*Pseudomonas* spp.	98	DQ084461	2	1	–	–
22P	f	1	*Rahnella aquatilis*	99	AY253919	3	3	–	–
20P	d	1	*Serratia proteamaculans*	100	AY040208	4	1	–	–

[a] RAPD-PCR profile, species identification by sequencing of the 16S rRNA gene, growth at different temperatures, and proteolytic activity at 7°C are indicated.
[b] Number of days to obtain growth on PCA at a defined temperature. –, no growth.

philes and *C. maltaromaticum* 9P, *Pseudomonas* spp. strain 28P, and *P. fragi* 25P among the psychrotrophs were able to give a clear zone of proteolysis around the wells on agar plates after destaining of Coomassie blue-colored plates (Tables 1 and 2).

Inoculated meat storage under vacuum at 7°C. The results of the viable counts of the meat chops contaminated with the selected strains and stored under vacuum at 7°C are shown in Table 3. The initial removal of the external part of the meat, performed to achieve decontamination, resulted in a total microbial load lower than 10^3 CFU g^{-1}, with viable counts ranging from 10 to 10^2 CFU g^{-1} of the targeted microbial groups (Table 3). The initial contamination of the artificially spiked chops was between 10^4 and 10^5 CFU g^{-1} for all of the strains inoculated singly or in a mix. In the control sample, all of the targeted microbial groups reached values above 10^4 CFU g^{-1} at the end of storage. In the contaminated samples the initial load of the inoculated bacteria increased during the 30 days of refrigerated storage, with the highest increase shown by the carnobacteria (Table 3). Random isolates from countable plates for each series of samples were subjected to DNA extraction and RAPD-PCR analysis as described above, and the

TABLE 3. Viable counts of spoilage microbial groups from meat chops inoculated with *S. proteamaculans* 42M, *P. fragi* 25P, and *C. maltaromaticum* 9P and stored at 7°C for 30 days

Medium	Time (days)	Mean viable counts (log CFU g^{-1}) ± SD[a]				
		42M	25P	9P	Mix	Control
VRBGA	0	4.84 ± 0.02^{A}	ND	ND	4.41 ± 0.01^{A}	1.20 ± 0.17^{A}
	5	5.00 ± 0.05^{A}	ND	ND	5.52 ± 0.02^{B}	2.11 ± 0.05^{B}
	15	5.50 ± 0.02^{B}	ND	ND	5.30 ± 0.15^{B}	5.05 ± 0.02^{C}
	30	6.95 ± 0.12^{C}	ND	ND	6.08 ± 0.13^{C}	5.10 ± 0.10^{C}
Pseudomonas agar	0	ND	4.92 ± 0.11^{A}	ND	4.86 ± 0.04^{A}	2.18 ± 0.02^{A}
	5	ND	5.70 ± 0.12^{B}	ND	6.23 ± 0.18^{B}	3.96 ± 0.01^{B}
	15	ND	5.75 ± 0.02^{B}	ND	5.95 ± 0.04^{B}	4.45 ± 0.03^{C}
	30	ND	6.05 ± 0.03^{B}	ND	6.24 ± 0.04^{B}	4.48 ± 0.04^{C}
CTSI	0	ND	ND	5.41 ± 0.11^{A}	4.70 ± 0.02^{A}	2.05 ± 0.12^{A}
	5	ND	ND	6.96 ± 0.10^{B}	4.63 ± 0.11^{A}	3.63 ± 0.04^{B}
	15	ND	ND	7.91 ± 0.06^{C}	4.05 ± 0.08^{B}	3.57 ± 0.03^{B}
	30	ND	ND	8.18 ± 0.06^{C}	3.78 ± 0.04^{B}	4.61 ± 0.05^{C}
PCA	0	5.00 ± 0.10^{A}	4.43 ± 0.04^{A}	5.41 ± 0.09^{A}	5.04 ± 0.14^{A}	2.56 ± 0.05^{A}
	5	5.25 ± 0.02^{A}	5.90 ± 0.03^{B}	6.96 ± 0.09^{B}	6.84 ± 0.13^{B}	5.40 ± 0.02^{B}
	15	6.96 ± 0.22^{B}	7.36 ± 0.08^{C}	7.91 ± 0.07^{C}	7.20 ± 0.08^{B}	5.63 ± 0.14^{B}
	30	8.12 ± 0.12^{C}	7.94 ± 0.09^{C}	8.18 ± 0.16^{C}	8.17 ± 0.07^{C}	5.10 ± 0.02^{B}

[a] For each culture medium, mean values with different superscripts in the same column are significantly different ($P < 0.05$). ND, not determined.

TABLE 4. Volatile organic compounds detected by GC-MS after 0, 15, and 30 days of storage at 7°C of meat chops inoculated with *S. proteamaculans* 42M, *P. fragi* 25P, and *C. maltaromaticum* 9P

Compound	Control meat inoculated at day:		Meat inoculated at day 0, 15, or 30 (as indicated) with[a]:											
			S. proteamaculans 42M			*C. divergens* 9P			*P. fragi* 25P			A mix of species		
	0	30	0	15	30	0	15	30	0	15	30	0	15	30
Alcohols														
3-Methyl-1-butanol					+									
2-Hexen-1-ol					+									
1-Octen-3-ol				+	+		+		t	+	+		t	+
2-Octen-1-ol					+		+							
2-Nonen-1-ol				+	+									
2-Hexyl-1-octanol						t	+							+
2-Methyl-1-dodecanol						t					t	+	+	+
2-Hexyl-1-decanol						t	+	+			+			+
2-Ethyldodecanol						t	+				+			+
2-Butyl-1-octanol							+	+		+	+		+	+
1-Dodecanol							+	+		+	t			
4-Methyl-1-dodecen-3-ol							+							
2-Methyl-1-undecanol							+							+
ter-Butylcyclohexanol							+							
Isotridecanol							+							
2-Buten-1-ol							+							
2-Ethyl-1-hexanol							+	+				t	+	+
5-Methyl-1,5-hexadien-3-ol								+						
1-Undecanol								+						
Hexadecanol								+						+
1,9-Nonandiol											+			
6-Dodecenol											+			
Hexadecandiol											+			
2-Pentadecanol											+			
2-Methyl-1-decanol										+	+		+	+
2-Methyl-3-buten-1-ol										+	+			
1-Nonanol										+	+		+	
2-Undecanol											+			
1,2-Dodecandiol										+	+			
5-Octen-2-ol											+			
2-Ethyl-1,3-hexandiol										+				
2-Ethyl-1-decanol										+				+
Tetradecen-1-ol												+	+	
1-Nonen-3-ol													+	
2-Dodecanol													+	
6-Methyl-1-octanol														+
Aldehydes														
Hexanal	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4-Idroxy-3-methylbutanal				+	+									
Nonanal							+	+	t	+	+		t	+
Decanal							+				+			+
2-Ethyl hexanal							+							
Tetradecanal							+							
2-Ethenylbutenal								+						
7-Hexadecenal								+						
2-Butyl octenal											+			
10-Undecenal											+			
5-Dodecenal														+
Dodecenal														+
Tridecanal											+			
Ketones														
2-Nonanone							+							
3-Octanone					+									
5-Methyl-4-hepten-3-one					+									
2-Octen-2-one										+	+			
2,5-Octandione										+	+			
2-Methyl-3-decen-5-one											+			
3-Decen-2-one											+			
2-Methyl-3-decen-5-one										+		+	+	

Continued on following page

TABLE 4—*Continued*

Compound	Control meat inoculated at day:		Meat inoculated at day 0, 15, or 30 (as indicated) with[a]:											
			S. proteamaculans 42M			*C. divergens* 9P			*P. fragi* 25P			A mix of species		
	0	30	0	15	30	0	15	30	0	15	30	0	15	30
Esters														
Ethyl hexanoate					+		+							
Ethyl octanoate			t		+		+	t						+
Ethyl nonanoate					+									
Ethyl decanoate					+									+
2-Hexen-1-ol propanoate				+	+									
Isoamyl acetate					+									
2-Ethylhexyl-2-ethyl hexanoate							+							
2-Tetradecylmethoxyacetate							+							
9,12-Tetradecadien-1-ol acetate							+							
Terbutyl cyclohexyl acetate							+							
Ethenyl decanoate								+						
Hexyl formiate										+	+		+	
Dodecyl hexanoate														+
Decenyl acetate														+
Tetradecen-1-ol acetate														+
Hydrocarbons														
9-Methyl-2-decene	t	t	t	t	t	+	t	t	+	t	t	+	+	
4-Dodecene		+												
5-Butyl-4-nonene	t	+	t	t	t	+	+	t	+	+	+	+	+	+
2-Methyl-2-decene	t	t	t	t	t	+	t	t	+	t	+	+	+	
2-Methyl-2-dodecene							+		+	+	+	+	+	+
Decane						+	+	+						
2-Dodecene							+							
1-Tetradecene							+	+				+	+	+
3-Tridecene								+						
5-Undecene								+						
8-Methyl-1-undecene								+						
5-Octadecene								+						
9-Octadecene								+						
5-Methyl undecene											+			
3-Tetradecene											+			
Undecene											+			
Dodecene											+			
1-Butene										+				
2,4,4-Trimethyl-1-pentene										+				
2-Decene										+				
1-Hexadecene														+
4-Methyl undecene													+	
Aromatic hydrocarbons														
Toluene	+	+	t	+	+	+	+	+	t	+	+	t	t	+
p-Dimethylbenzene	+	+	+	+	+	+	+	+	+	+	+	+	+	+
o-Dimethylbenzene	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1-Methyl-2,1-methylethylbenzene					+									
Terpene compounds														
Caryophyllene	+													
Citronellyl acetate	+	+	+	+	+	t	+	+	+	t	+	+	+	t
Limonene	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tujolo		+												
Linalool			t	+	+		+			t				
Linalyl propanoate					+									
Menthol				+	+		+	+					+	
Borneol				+										
α-Pinene														+
2,3-Epoxy geranyl acetate														+
α-Terpineol				+							+			
Isobornyl acetate							+	+						
Limonene oxide							+							

Continued on following page

TABLE 4—*Continued*

Compound	Control meat inoculated at day:		Meat inoculated at day 0, 15, or 30 (as indicated) with[a]:											
			S. proteamaculans 42M			*C. divergens* 9P			*P. fragi* 25P			A mix of species		
	0	30	0	15	30	0	15	30	0	15	30	0	15	30
Phenols														
4-Methylguaiacol	+	+	+	+	+	+	+	+	+	t	+	+	+	t
Butylhydroxytoluene	+	t	t	+	t	+	+	+	+	+	+	+	+	+
4-Methoxybenzhydrol	+	+	t	+	+	+	+	+	+	t	+	+	+	+
4(1,1,3,3)-Tetramethylbutyl)phenol									+					
2-Ethylphenol												+	+	
Sulfur compounds														
Carbon disulfide	t		t			t	+	+		+	+	+	+	+
1-Propanthiol		t		+	+		+	+						
2-Pentyl thiophene													+	+
Methylethylsulfide					+									
4-Methylthiophenol								+						
Undecanthiol								+						
Methoxybenzenthiol								+						
2-Methyl undecanthiol											+			
Lactone														
δ-Nonalactone							+							
Phtalate														
Dibutylphtalate						t	+	t	t	+	t	t	+	+
Organic acids														
Tetradecenoic acid								+						
3-Hydroxydodecanoic acid														+

[a] t, molecule occurs in trace amounts.

identity was confirmed between isolates and corresponding inoculated biotype (results not shown).

VOC determination during storage. The results of the VOC analysis of the samples during the storage of meat are reported in Table 4. Overall, the uncontaminated control samples displayed a reduced diversity and amount of volatile compounds compared to the inoculated samples. Some metabolites (e.g., 2-hexyl-1-octanol and 2-hexyl-1-decanol) were detected already at time zero, and since they did not occur in the control samples they likely arose from the residues of growth media of the cultures used for the contamination (Table 4). In all of the contaminated samples the diversity of the VOC and their concentration increased during the time of storage. This is particularly evident for samples contaminated by *S. proteamaculans* 42M; some molecules, such as 1-octen-3-ol, ethyl decanoate, and isoamyl acetate, were detected only after 30 days, while ethyl octanoate, present only in minimal traces at time zero, increased during the storage time (Fig. 1).

Qualitative data reported in Table 4 indicated that each contamination resulted in complex and specific VOC profiles of the meat during the chill storage, and the diversity of the samples contaminated by the different cultures after 30 days of

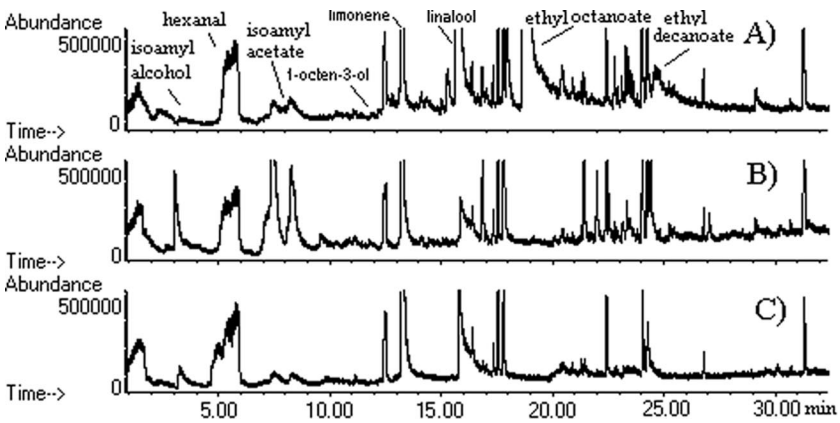

FIG. 1. Total ion current chromatograms obtained for meat samples contaminated by *S. proteamaculans* M42 by HS-SPME-GC/MS analysis. (A) 42M, after 30 days of storage (t30); (B) 42M, t15; (C) 42M, t0.

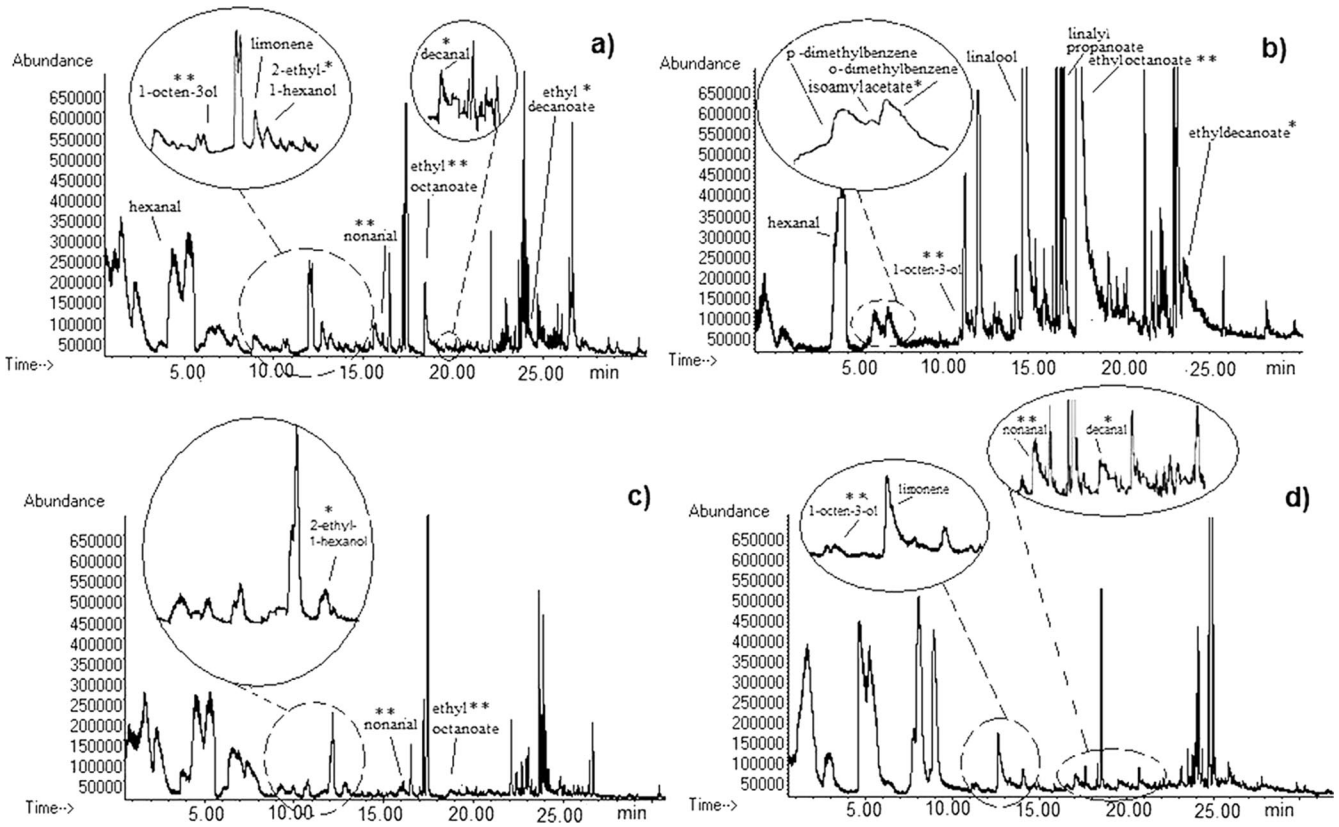

FIG. 2. Total ion current chromatograms obtained for Mix t30 (a), 42M after 30 days of storage (t30) (b), 9P t30 (c), and 25P t30 (d) samples by HS-SPME-GC/MS analysis. * and **, quantified volatile metabolites detected at 30 days for only one and two species, respectively, among the three considered.

storage is shown in Fig. 2. Beef chops contaminated with *S. proteamaculans* 42M were characterized by the presence of alcohols such as 1-octen-3-ol, and some esters, such as ethyl hexanoate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, and isoamyl acetate, at the end of the storage time (Table 4 and Fig. 2b). In addition, 1-propanthiol was detected after 15 and 30 days. The samples contaminated by *P. fragi* 25P displayed the highest diversity in ketones and higher-molecular-weight alcohols after 15 days of storage (Table 4). The same samples did not show the presence of esters except for ethyl formiate; in addition, some aldehydes such as 2-buthyl-octenale and 10-undecenal, occurred only in these beef samples (Table 4). The meat contaminated by *C. maltaromaticum* 9P was the richest in alcohols displaying 17 different molecules, 13 of which were detected after 15 days and 7 of which were detected after 30 days, respectively (Table 4). Some of these alcohols, such as 2-ethyl-1-hexanol, 2-buten-1-ol, and 2-hexyl-1-octanol, along with 2-nonanone and 2-ethylhexanal, were not present in the other contaminated samples (Table 4 and Fig. 2c). Carbon disulfide and 1-propanthiol also generated during the storage of meat spiked with *C. maltaromaticum* 9P. Most of the molecules occurring in the samples contaminated with the mix of strains could also be detected in the samples contaminated by a single strain (Table 4). However, in the mix-inoculated samples, a general decrease of metabolites was observed in comparison to the other inoculated samples (Fig. 2 and Fig. 3).

Some volatiles detected in the mix-inoculated samples could be considered strain molecular markers since they were detected only in meats contaminated by one strain among the three considered (Fig. 2 and Table 4). Among others, 2-ethyl-1-hexanol was detected, in addition to the mix-inoculated sample, only in samples contaminated by *C. maltaromaticum* 9P; decanal was found only in 25P- and mix-inoculated samples; and ethyl decanoate was detected only in 42M- and mix-inoculated samples (Fig. 2). In addition, 2-nonanone and 2-ethylhexanal were observed only in the 9P-inoculated samples at 15 days, which is also the sample with the highest quantity of 2-ethyl-1-hexanol (Table 4 and Fig. 3). Isoamyl acetate was detected only in the 42M-inoculated sample at 30 days, which is also the sample with the highest quantity of isoamyl alcohol (3-methyl-1-butanol) (Fig. 2 and Table 4). A cumulative effect in the production of volatile metabolites by the strains in the mix-inoculated samples, and a consequent increase of their related detected quantities, appeared only for some compounds. For example, the quantity of nonanal was higher in the mix-inoculated than in the singly inoculated samples at 30 days (Fig. 2). Moreover, among 3-hydroxy straight-chain fatty acids, which occur in *Pseudomonas* and *Serratia* spp. as major acyl component of lipolysaccharides in cell walls (31), the 3-hydroxydodecanoic acid was detected only in the mix at 30 days, possibly as a result of its production by both species in the mix (Table 4).

Quantitative indications obtained for some representative

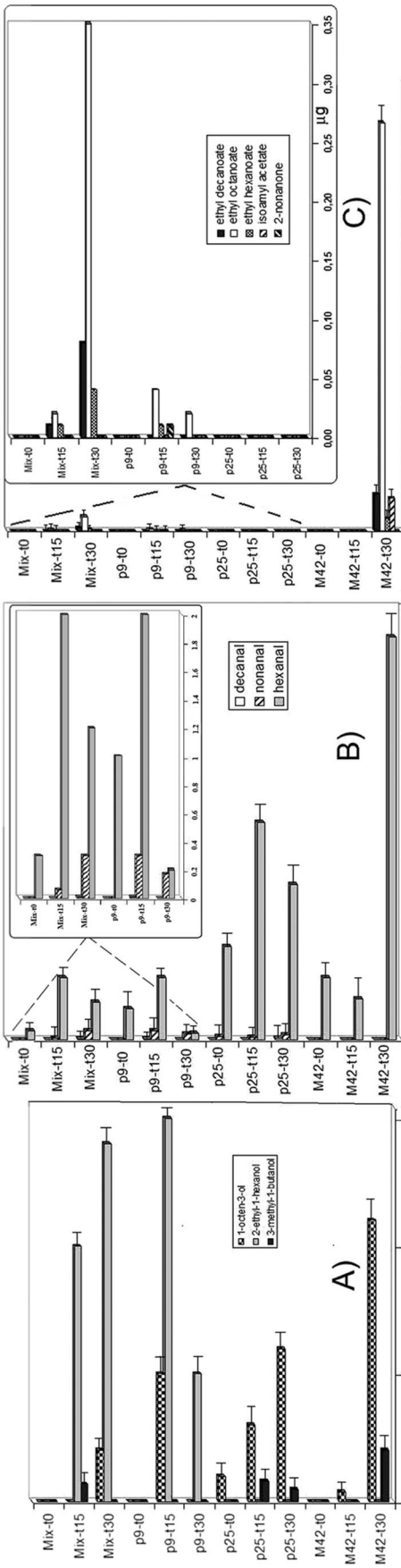

FIG. 3. Quantitative indications (mean values, µg/5 g of contaminated meat samples) of some representative volatile metabolites, including alcohols (1-octen-3-ol; 2-ethyl-1-hexanol, and 3-methyl-1-butanol) (A), aldehydes (hexanal, nonanal, and decanal) (B), and carbonilic compounds (isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and 2-nonanone) (C).

volatile metabolites are reported in Fig. 3. During the storage time, an increase in the content of these volatile metabolites was observed for the analyzed meat samples. However, for some samples a decrease in the content of some metabolites was observed between 15 and 30 days. For example, 2-ethyl-1-hexanol, 1-octen-3-ol, hexanal, nonanal, 2-nonanone, and ethyl esters decreased in 9P-inoculated samples between 15 and 30 days (Fig. 3).

DISCUSSION

In this work, the microbial diversity of mesophilic and psychrotrophic bacteria isolated from vacuum packaged beef was evaluated. The isolates were identified by 16S rRNA gene sequencing and biotyped by RAPD-PCR. For the numbering and isolation, a nonselective medium such as PCA was used, which is commonly used for the cultivation of mesophilic and psychrotrophic bacteria from foods (29). A considerable microbial diversity, including enterobacteria, pseudomonads, and carnobacteria, was found in both mesophilic and psychrotrophic groups of microorganisms, including taxa often recognized as spoilage agents (4, 14, 15, 21, 25, 33, 56, 58). *C. maltaromaticum* and *C. divergens*, recognized as dominant spoilers of meat (35), were isolated at both 30 and 7°C. *Pseudomonas* spp., except *P. putida* 39M, were commonly isolated only at 7°C, while enterobacteria were mainly isolated at 30°C. The biotype screening by RAPD-PCR was initially performed in order to reduce the number of isolates to be identified by sequencing. However, the results of the RAPD-PCR analysis of carnobacteria isolates highlighted that different biotypes of the same species were isolated as mesophilic or psychrotrophic bacteria, indicating that they may adapt to grow at very different temperatures. A high diversity was also noticed as result of the trials of growth at different temperatures, where the same species displayed different behaviors of growth at 7, 20, and 30°C. Overall, the mesophilic bacteria grew fast at 30 and 20°C and within 5 days at 7°C. In contrast, the psychrotrophic bacteria grew slowly or did not grow at all at 30°C, but most of the strains grew rapidly at 20°C and within 3 days at 7°C. Jay (29) proposed that, until general agreement can be reached, the psychrotrophs from meat and poultry products could be isolated after incubation at 7°C for 10 days. Our results corroborate this suggestion with the evidence that the main difference between the growth capability of mesophilic and psychrotrophic bacteria is the fact that the latter are disadvantaged in the growth at 30°C.

Although the role of bacteria in the development of spoilage-related molecules during meat storage is recognized, there is still a lack of available data on the VOC profiles of meat during storage and on the possible link to the microbial species acting as spoilage agents. Therefore, a further aim of the present study was to investigate the species-dependent composition of VOC released in meat during storage. Since the spoilage potential of bacteria is mainly related to their proteolytic activity, we chose the proteolytic isolates described above for the meat contamination and VOC monitoring experiments.

About 100 volatile molecules were detected in the HS of the analyzed samples. Although the analyses were performed on samples with an initial background microflora of about 10^2 CFU/g after decontamination, this microbiota did not affect

the spoilage of meat with the release of VOC as its profiles were poor in molecules. Therefore, it can be assumed that the volatiles detected in the profiles of meat inoculated with the selected strains were produced by the strains themselves with no influence from the background microbial flora. There are several possible origins for these volatile metabolites, and the range of organic compounds that can be used as carbon and energy source by these bacteria is wide. Although it is difficult, and in some cases not possible, to attribute them to a specific biosynthetic pathway, some hypotheses can be made. 3-Methyl-1-butanol (isoamyl alcohol, which was present in an M42-inoculated sample at 30 days also as isoamyl acetate) could derive from the proteolytic activity of the strains and from leucine catabolism (57). In fact, small-branched alcohols and aldehydes are key intermediates in the branched-chain amino acid catabolism, which can serve various purposes, such as the formation of branched-chain fatty acids for cell membrane synthesis in several microbial species (3, 44, 57). Sulfur compounds usually arise from sulforated amino acids catabolism (2, 44). Some methyl ketones such as 2-nonanone can derive from a lypolytic process but also from several other possible pathways, such as alkane degradation by *Pseudomonas* through a unique alpha-oxidation, with no change in the carbon skeleton (51, 59). Methylketones are also formed by bacterial dehydrogenation of secondary alcohols, a reaction that appears to be part of the alkane oxidation sequence (37, 51). Ethyl esters were formed through chemical esterification of alcohols and carboxylic acids following microbial esterase activity (60, 61). Carboxylic acids deriving from hydrolysis of triglycerides and phospholipids (61) can also be an initial substrate for oxidation with the formation of final odorous products. For example, 2-nonanone, among the different possible metabolic pathways, can be formed through the beta-oxidation of decanoic acid derived from lipolysis and a in second step through beta-ketoacid decarboxylation (44). Aldehydes, such as hexanal, nonanal, and decanal, and also alcohols, such as 1-octen-3-ol, can derive from hydrolysis of triglycerides or from amino acid degradation (44). Some terpenols (such as linalool and some related compounds such as linalyl propanoate) appeared more abundant and frequent in 42M-inoculated samples compared to 9P- and 25P-contaminated samples (Fig. 1 and Table 4). These differences could be also related to possible different metabolic pathways: it has been reported that terpenols (and long branched-chain alcohols also) can be degraded by some species of *Pseudomonas*, yielding metabolites identical to those found in the amino acid-specific pathways (41, 59). Butylhydroxytoluene and 4-methylguaiacol usually derive from animal feeding (6), and phtalates can arise from packaging, while thiophenes derive from proteins and phospholipids (22).

Among the detected volatile molecules, some possible specific markers were identified. Compounds such as 2-ethyl-1-hexanol, 2-buten-1-ol, 2-hexyl-1-octanol, 2-nonanone, 2-ethylhexanal, for example, were detectable only for *C. maltaromaticum*, which produced also the highest number of aldehydes, lactones, and sulfur compounds, while the highest number of alcohols and ketones were detected in the HS of meat samples contaminated by *P. fragi*, and the highest concentrations of some alcohols (such as 1-octen-3-ol) and some esters (such as isoamyl acetate) were produced by *S. proteamaculans*. As a matter of fact, for a particular molecule to be regarded as a marker of spoilage development by a certain species, it should be produced by that species regardless of the strain. Therefore, further studies are necessary to investigate the release of volatile compounds in meat by different strains of the species analyzed here in order to support the possibility of using some of the metabolites as spoilage tracers.

Several volatile compounds found in the present study were also recently detected by SPME-GC/MS in cooked ham stored at 7°C in modified atmosphere packaging (36). Only a few esters were found in the meat samples inoculated with *P. fragi* 25P, although several studies report on the esters as the major volatile compounds produced by *P. fragi* in spoiling foods (8, 13, 45). However, Edwards et al. (13) evidenced that not all of the strains of *P. fragi* that they used to spoil meat at a chill temperature were able to produce esters. In addition, when six different species of *Pseudomonas* were screened for their activity in cheese, it was shown that the production of volatile compounds is species dependent (46). In our case, esters were detected in samples contaminated by *S. proteamaculans* and *C. maltaromaticum*; several of them were also found in cured ham spoiled by enterobacteria (20).

The VOC profiles obtained in the present study were complex, especially after the first 15 days of storage; therefore, a complexity of flavor release from our samples can be hypothesized. We did not use sniffing or sensory analysis in order to associate molecule identification with a possible role as off-odors. However, several detected volatiles can be odor-active molecules, with odor threshold ranging between some units and several tens of ppm depending on the specific texture interactions, which in meat tend to increase in comparison to water (44). Some of the detected molecules are indicated to cause possible off-odor in meat. 3-Methyl-1-butanol can be associated with a whiskey-like odor (34). High quantities of hexanal (odor threshold in meat 0.015 ppm) can produce rancid, green leave off-flavor. Nonanal (pelargonium, rancid odor descriptor, threshold in oil of 1 ppm) (39) and 1-octen-3-ol (odor descriptor mushroom, threshold in oil of 0.0075 ppm) were also related to an unpleasant sensory answer (6).

Studies based on MS techniques are still instrumental in understanding the basic metabolism of meat-associated bacteria. However, these kinds of studies may help to provide useful data for the interpretation of responses obtained from electronic nose analyses applied in the quality assurance and quality control for meat products. SPME-GC/MS has been often used for the analysis of cured or cooked meat products (22, 36, 38, 50, 52). However, there is still a lack of available data on the chemical composition and structural identity of the volatile metabolites in the HS of raw meat samples kept under controlled conditions that can be related to species-specific microbial metabolism. Other authors have exploited proton transfer reaction-MS for the detection of VOC during meat spoilage (43), to measure the effect of ozone treatment for pork preservation (27), and have tried to correlate the viable counts with the volatile detection for an effective prediction of the spoilage dynamics (43). According to our results, the volatile profiles may change according to the species and perhaps biotype development, making a monitoring of the spoilage much more difficult by VOC determination. Moreover, we demonstrated that the combination of different species may or may not result in a combined release of VOC because of species interaction

during meat storage. The proton transfer reaction-MS technique has been recently proposed for the on-line monitoring of volatile compounds produced by several bacteria (5), and it would be interesting to exploit this application to differently contaminated meats.

For a better understanding of spoilage development, it is important to evaluate whether all of the microbial species and biotypes are equally responsible for the chemical changes associated to the spoilage of meat. Moreover, the availability of spoilage molecular markers could be useful for an effective analysis of the spoilage dynamics in studies aimed at developing the most suitable conditions for the prevention of quality loss and for the extension of fresh meat shelf-life.

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REFERENCES

1. **Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
2. **Ardö, Y.** 2006. Flavour formation by amino acid catabolism. Biotechnol. Adv. **24:**238–242.
3. **Beck, H. C., A. M. Hansen, and F. R. Lauritsen.** 2004. Catabolism of leucine to branched-chain fatty acids in *Staphylococcus xylosus*. J. Appl. Microbiol. **96:**1185–1193.
4. **Borch, E., M.-L. Kant-Muermans, and Y. Blixt.** 1996. Bacterial spoilage of meat and cured meat product. Int. J. Food Microbiol. **33:**103–120.
5. **Bunge, M., N. Araghipour, T. Mikoviny, J. Dunkl, R. Schnitzhofer, A. Hansel, F. Schinner, A. Wisthaler, R. Margesin, and T. D. Märk.** 2008. On-line monitoring of microbial volatile metabolites by proton transfer reaction-mass spectrometry. Appl. Environ. Microbiol. **74:**2179–2186.
6. **Calkins, C. R., and J. M. Hodgen.** 2007. A fresh look at meat flavour. Meat Sci. **77:**63–80.
7. **Chung, K.-T., J. S. Dickson, and J. D. Crouse.** 1989. Attachment and proliferation of bacteria on meat. J. Food Prot. **52:**173–177.
8. **Cormier, F., Y. Raymond, C. P. Champagne, and A. Morin.** 1991. Analysis of odor-active volatiles from *Pseudomonas fragi* grown in milk. J. Agric. Food Chem. **39:**151–161.
9. **Costerson, J. W., R. T. Irvin, and K.-J. Cheng.** 1981. The bacterial glycocalyx in nature and disease. Annu. Rev. Microbiol. **35:**299–324.
10. **Dainty, R. H., B. G. Shaw, and T. A. Roberts.** 1983. Microbial and chemical changes in chill stored red meats, p. 151–178. *In* T. A. Roberts and F. A. Skinner (ed.), Food microbiology: advances and prospects. Academic Press, London, England.
11. **Dainty, R. H., and B. M. Mackey.** 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. J. Appl. Bacteriol. **73:**103S–114S.
12. **Di Maro, E., D. Ercolini, and S. Coppola.** 2007. Yeast dynamics during spontaneous wine fermentation of the Catalanesca grape. Int. J. Food Microbiol. **117:**201–210.
13. **Edwards, R. A., R. H. Dainty, and C. M. Hibbard.** 1987. Volatile compounds produced by meat pseudomonads and related reference strains during growth on beef stored in air at chill temperatures. J. Appl. Bacteriol. **62:**403–412.
14. **Ercolini, D., F. Russo, E. Torrieri, P. Masi, and F. Villani.** 2006. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. Appl. Environ. Microbiol. **72:**4663–4671.
15. **Ercolini, D., F. Russo, G. Blaiotta, O. Pepe, G. Mauriello, and F. Villani.** 2007. Simultaneous detection of *Pseudomonas fragi*, *P. lundensis*, and *P. putida* from meat by a multiplex PCR assay targeting the *carA* gene. Appl. Environ. Microbiol. **73:**2354–2359.
16. **Ercolini, D., F. Russo, I. Ferrocino, and F. Villani.** 2009. Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk. Food Microbiol. doi:10.1016/j.fm.2008.09.005.
17. **Ercolini, D., G. Frisso, G. Mauriello, F. Salvatore, and S. Coppola.** 2008. Microbial diversity in natural whey cultures for the production of Caciocavallo Silano PDO cheese. Int. J. Food Microbiol. **124:**164–170.
18. **Firstenberg-Eden, R.** 1981. Attachment of bacteria to meat surfaces: a review. J. Food Prot. **44:**602–607.
19. **Fung, D. W. C., J. Edwards, and B. A. Crozier-Dodson.** 2008. At-line methods for controlling spoilage, p. 289–318. *In* F. Toldrà (ed.), Meat biotechnology. Springer, New York, NY.
20. **García, C., A. Martín, M. L. Timón, and J. J. Córdoba.** 2000. Microbial populations and volatile compounds in the "bone taint" spoilage of dry cured ham. Lett. Appl. Microbiol. **30:**61–66.
21. **Gill, C. O., and K. G. Newton.** 1978. The ecology of bacterial spoilage of fresh meat at chill temperatures. Meat Sci. **14:**43–60.
22. **Hansen, T., M. A. Petersen, and D. V. Byrne.** 2005. Sensory based quality control utilizing an electronic nose and GC-MS analyses to predict end-product quality from raw materials. Meat Sci. **69:**621–634.
23. **Hitchener, B. J., A. F. Egan, and R. J. Rogers.** 1982. Characteristics of lactic acid bacteria isolated from vacuum packaged beef. J. Appl. Bacteriol. **52:**31–37.
24. **Holzapfel, W. H.** 1998. The gram-positive bacteria associated with meat and meat products, p. 35–74. *In* A. Davies and R. Board (ed.), The microbiology of meat and poultry. Blackie Academic & Professional, London, England.
25. **Huis in't Veld, J. H. J.** 1996. Microbial and biochemical spoilage of foods: an overview. Int. J. Food Microbiol. **33:**1–18.
26. **Jackson, T. C., G. R. Acuff, and J. S. Dickson.** 1997. Meat, poultry, and seafood, p. 83–100. *In* M. P. Doyle and L. R. Beuchat (ed.), Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC.
27. **Jaksch, D., R. Margesin, T. Mikoviny, J. D. Skalny, E. Hartungen, F. Schinner, N. J. Mason, and T. D. Märk.** 2004. The effect of ozone treatment on the microbial contamination of pork meat measured by detecting the emissions using PRT-MS and by enumeration of microorganisms. Int. J. Mass Spectrom. **239:**209–214.
28. **Jay, J. M.** 2000. Food preservation with modified atmospheres, p. 283–295. *In* D. R. Heldman (ed.), Modern food microbiology. Aspen Publishers, Inc., Frederick, MD.
29. **Jay, J. M.** 2002. A review of aerobic and psychrotrophic plate count procedures for fresh meat and poultry products. J. Food Prot. **65:**1200–1206.
30. **Jay, J. M., J. P. Vilai, and M. E. Hughes.** 2003. Profile and activity of the bacterial biota of ground beef held from freshness to spoilage at 5–7°C. Int. J. Food Microbiol. **81:**105–111.
31. **Kaneda, T.** 1991. Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol. Rev. **55:**288–302.
32. **Koutsoumanis, K., A. Stamatiou, P. Skandamis, and J.-G. Nychas.** 2006. Development of microbial model of temperature and pH on spoilage of ground beef, and validation of the model under dynamic temperature conditions. Appl. Environ. Microbiol. **72:**124–134.
33. **Labadie, J.** 1999. Consequences of packaging on bacterial growth: meat is an ecological niche. Meat Sci. **52:**299–305.
34. **Larrouture-Thiveyrat, C., M. Pepin, S. Leroy-Sétrin, and M.-C. Montel.** 2003. Effect of *Carnobacterium piscicola* on aroma formation in sausage mince. Meat Sci. **63:**423–426.
35. **Laursen, B. G., L. Bay, I. Cleenwerck, M. Vancanneyt, J. Swings, P. Dalgaard, and J. J. Leisner.** 2005. *Carnobacterium divergens* and *Carnobacterium maltaromaticum* as spoilers or protective cultures in meat and seafood: phenotypic and genotypic characterization. Syst. Appl. Microbiol. **28:**151–164.
36. **Leroy, F., C. Vasilopoulos, S. Van Hemelryck, G. Falony, and L. De Vuyst.** 2009. Volatile analysis of spoiled, artisan-type, modified-atmosphere-packaged cooked ham stored under different temperatures. Food Microbiol. doi:10.1016/j.fm.2008.08.005.
37. **Lukins, H. B., and J. W. Foster.** 1963. Methyl ketone metabolism in hydrocarbon-utilizing mycobacteria, J. Bacteriol. **85:**1074–1087.
38. **Marco, A., J. L. Navarro, and M. Flores.** 2004. Volatile compounds of dry-fermented sausages as affected by solid-phase microextraction (SPME). Food Chem. **84:**633–641.
39. **Marco, A., J. L. Navarro, and M. Flores.** 2007. Quantitation of selected odor-active constituents in dry fermented sausages prepared with different curing salts. J. Agric. Food Chem. **55:**3058–3065.
40. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3:**208–218.
41. **Massey, L. K., J. R. Sokatch, and R. S. Conrad.** 1976. Branched-chain amino acids catabolism in bacteria. Bacteriol. Rev. **40:**42–54.
42. **Mauriello, G., A. Casaburi, and F. Villani.** 2002. Proteolytic activity of *Staphylococcus xylosus* strains on pork myofibrillar and sarcoplasmic proteins and use of selected strains in the production of "Naples type" salami. J. Appl. Microbiol. **92:**482–490.
43. **Mayr, D., R. Margesin, E. Klingsbichel, E. Hartungen, D. Jenewein, F. Schinner, and T. D. Märk.** 2003. Rapid detection of meat spoilage by measuring volatile organic compounds by using proton transfer reaction-mass spectrometry. Appl. Environ. Microbiol. **69:**4697–4705.
44. **Montel, M. C., F. Masson, and R. Talon.** 1998. Bacterial role in flavour development. Meat Sci. **49:**S111–S123.
45. **Morales, P., E. Fernandez-Garcia, and M. Nunez.** 2005. Production of volatile compounds in cheese by *Pseudomonas fragi* strains of dairy origin. J. Food Prot. **68:**1399–1407.
46. **Morales, P., E. Fernandez-Garcia, and M. Nunez.** 2005. Volatile compounds produced in cheese by *Pseudomonas* strains of dairy origin belonging to six different species. J. Agric. Food Chem. **53:**6835–6843.

47. **Nychas, G.-J. E., and E. H. Drosinos.** 1999. Meat and poultry spoilage, p. 1253–1259. *In* R. K. Robinson, C. A. Batt, and P. D. Patel (ed.), Encyclopedia of food microbiology. Academic Press, Inc., San Diego, CA.
48. **Nychas, G.-J. E., E. H. Drosinos, and R. G. Board.** 1998. Chemical changes in stored meat, p. 289. *In* A. Davies and R. Board (ed.), The microbiology of meat and poultry. Blackie Academic & Professional, London, England.
49. **Nychas, G.-J. E., P. Skandamis, C. C. Tassou, and K. P. Koutsoumanis.** 2008. Meat spoilage during distribution. Meat Sci. **78:**77–89.
50. **O'Sullivan, M. G., D. V. Byrne, M. T. Jensen, H. J. Andersen, and J. Vestergaard.** 2003. A comparison of warmed-over flavour in pork by sensory analysis, GC/MS and the electronic nose. Meat Sci. **65:**1125–1138.
51. **Padda, R. S., K. K. Pandey, S. Kaul, V. D. Nair, R. K. Jain, S. K. Basus, and T. Chakrabarti.** 2001. A novel gene encoding a 54-kDa polypeptide is essential for butane utilization by *Pseudomonas* sp. IMT37. Microbiology **147:**2479–2491.
52. **Rivas-Cañedo, A., E. Fernández-García, and M. Nuñez.** 2009. Volatile compounds in fresh meats subjected to high pressure processing: effect of the packaging material. Meat Sci. **81:**321–328.
53. **Sanz, Y., S. Fadda, G. Vignolo, M.-C. Aristoy, G. Oliver, and F. Toldrà.** 1999. Hydrolysis of muscle myofibrillar proteins by *Lactobacillus curvatus* and *Lactobacillus sake*. Int. J. Food Microbiol. **53:**115–125.
54. **Shaw, B. G., and C. D. Harding.** 1984. A numerical taxonomic study of lactic acid bacteria from vacuum-packed beef, pork, lamb, and bacon. J. Appl. Bacteriol. **56:**25–40.
55. **Shaw, B. G., and C. D. Harding.** 1989. *Leuconostoc gelidum* sp. nov. and *Leuconostoc carnosum* sp. nov. from chill-stored meats. Int. J. Syst. Bacteriol. **39:**217–223.
56. **Skandamis, P., and G.-J. E. Nychas.** 2005. Fresh meat spoilage and modified atmosphere packaging, p. 461–493. *In* Raw material safety: meat. Woodhead Publishers, Cambridge, United Kingdom.
57. **Smit, G., B. A. Smit, and W. J. M. Engels.** 2005. Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. FEMS Microbiol. Rev. **29:**591–610.
58. **Stanbridge, L. H., and A. R. Davies.** 1998. The microbiology of chill-stored meat, p. 175–177. *In* A. Davies and R. Board (ed.), Microbiology of meat and poultry. Blackie Academic & Professional, London, England.
59. **Stanyer, R. Y., N. J. Palleroni, and M. Doudoroff.** 1996. The aerobic pseudomonads: a taxonomy study. J. Gen. Microbiol. **43:**159–271.
60. **Talon, R., C. Chastagnac, L. Vergnais, M. C. Montel, and J. L. Berdagué.** 1998. Production of esters by staphylococci. Int. J. Food Microbiol. **45:**143–150.
61. **Toldrà, F.** 1998. Proteolysis and lipolysis in flavour development of dry-cured meat products. Meat Sci. **49:**S101–S110.
62. **Wasney, M. A., R. A. Holley, and D. S. Jayas.** 2001. Cresol red thallium acetate sucrose inulin (CTSI) agar for the selective recovery of *Carnobacterium* spp. Int. J. Food Microbiol. **64:**167–174.
63. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173:**697–703.